Determination of Copper in Edible Soybean Oils[1]

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Abstract

Soybean oils have been analyzed for their copper content before and after hydrogenation with copper-containing catalysts. A low-temperature dry asher, an apparatus in which oxygen plasma is generated in a radio frequency field under high vacuum, was adopted for ashing glyceride oils. The residues were analyzed by a colorimetric procedure using zinc dibenzyldithiocarbamate as the reagent. Identical samples were analyzed without ashing by neutron activation and atomic absorption techniques. The accuracy of the methods was determined by adding known amounts of copper at four different levels to two different soybean salad oils. Plots of copper found versus added copper showed that results were consistent over the range 0.04–5.0 ppm for all three methods, but that the atomic absorption results were low. The relative error of a single determination was ±13% and that of the mean of duplicate determinations ±9%. Analysis of natural soybean oils showed a copper content of about 0.03–0.10 ppm, whereas the same oils hydrogenated with copper-containing catalysts and without metal removal treatments had levels of 3–5 ppm.

Introduction

Work at the Northern Laboratory (7,11,12,17), by Unilever (6), and in Japan (16) has shown that certain copper-containing catalysts are extremely selective for the reduction of linolenyl groups in soybean oil. These catalysts have potential for the production of oils with low linolenate content. The use of such catalysts in hydrogenating soybean oil has led to a re-examination of the problem of trace copper analysis in glyceride oils. Copper exerts a strong pro-oxidant effect in edible fats and as little as 0.03 ppm will quickly lower the flavor score of a soybean oil (9). Assaying for traces of copper in glyceride oils is difficult because this metal occurs in natural oils in extremely small amounts (8), usually at a few hundredth ppm. Direct extraction methods (2,13) have been proposed for copper determinations in petroleum oils, but they lack the sensitivity required for glyceride oils. Also, evidence exists that metals may occur in glyceride oils in a form not available for extraction by acids or chelating agents. Cooney et al. (5) proposed that metals may occur as a chelate between fat hydroperoxides and secondary oxidation products and that before acidic metal scavengers can be effective, a fat must be heated to decompose the peroxide-metal complex.

The usual method for trace copper analysis in biological materials is to react the copper with a specific reagent to form a colored complex which can be extracted and determined by visible spectrophotometry. For glyceride oils, an ashing step is required to obtain an aqueous solution suitable for analysis. Ashing may be accomplished in a number of ways, and these techniques have been reviewed by Snell and Snell (20). Strong mineral acids may be used, but our experience shows that some reagent grade acids contain significant metallic contaminants that make them unsuitable. Ashing at high temperatures may be used, but losses through volatility may occur. Recently a low-temperature dry asher has become available (21). In dry ashing, an oxygen plasma is generated within a radio frequency field under high vacuum, and this plasma serves as the oxidizing agent. Low-temperature ashing occurs anywhere from 80 C to 200 C. We have adopted this technique for ashing glyceride oils in our work. Although the rate of ashing is slow, no losses were encountered from volatility.

Many colorimetric reagents have been proposed for copper. Some include dithizone, cuproine and sodium diethyldithiocarbamate. These reagents and related analytical procedures have been reviewed by Sandell (18). Zinc dibenzyldithiocarbamate was first investigated by Martens and Githens (14) in 1952 who applied it as a colorimetric reagent for the determination of copper in dyes and rubber chemicals. Several years later Abbot and Polhill (1) adopted it for copper determination in sunflower and olive oils. Zinc dibenzyldithiocarbamate and other salts of dibenzyldithiocarbamic acids have certain advantages over other colorimetric reagents for copper. These include good stability in organic solvents, low interference from other metals and ready formation of light-stable complexes with copper.

Although ashing appears to be an essential prerequisite for colorimetric analyses of low copper levels, other analytical methods, particularly neutron activation (10) and atomic absorption, do not require ashing. These techniques, however, do involve elaborate and expensive equipment. Exclusive of the time needed for dry ashing, the colorimetric analyses described here took about 30 min. Dry ashing compares favorably, from the standpoint of simplicity, with any other method. Studies are reported on a combined low-temperature ashing and colorimetric technique used to determine the copper contents of edible soybean oil hydrogenated with copper-containing catalysts. Data are also presented for the same samples analyzed by neutron activation and atomic absorption techniques.

Experimental Procedures

The commercial soybean oils were either refined and bleached oils or refined, bleached and deodorized salad oils. The copper-hydrogenated soybean oil samples were prepared in either the laboratory or the pilot plant of the Northern Laboratory as described previously by Koritala and Dutton (12) and Moulton et al. (15). Zinc dibenzyldithiocarbamate was reagent grade (Aldrich Chemical Co.) and used without further purification. Since ordinary reagent grade sulfuric acid contained significant amounts of copper, it was not suitable in the colorimetric procedure. Sulfuric acid (95%) doubly distilled from Vycor (G. Fredrick Smith Chem. Co.) was low in metal content and gave very low copper blanks. Carbon tetrachloride was spectroquality. The low-

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temperature dry ashing apparatus used in this study was manufactured by the Tracerlab Corporation. The device is fitted with a five-chambered manifold along with vacuum and radio frequency gauges and the necessary controls for optimum ashing. Oxygen feed is controlled by a needle valve and monitored with a flowmeter in the system.

Dry Ashing

Steps in the dry ashing are as follows: Samples of oil (3–5 g) are accurately weighed to ±0.1 mg in ignition boats. The ignition boats are approximately 3½ × 1½ × ½ in. and have a capacity of about 20 g. (Samples larger than 5 g tend to spatter during degassing.) Sample boats are placed in the five-chambered manifold and a vacuum seal is made with greased glass plates. Vacuum is applied slowly to the system while the samples are being degassed. Final pressure within the system should be 0.05 mm Hg or less. The radio frequency field is generated and tuned to minimum reflected power. The discharge at this point is a brilliant blue. Oxygen is bled into the system so that a pressure of approximately 1 mm Hg is maintained. The radio frequency field is retuned to minimum reflected power. The apparatus can then be operated almost unattended with only an occasional tuning. Until oxidation is complete, the discharge is reddish in color.

Standard Absorption Curve

Copper turnings (100 mg, 99.9% purity) were dissolved in several milliliters of concentrated nitric acid and diluted to 100 ml with absolute ethanol. This stock solution contained 1,000 μg/ml of copper. One milliliter of the stock was diluted to 1,000 ml with water distilled twice in glass. Aliquots of the 1 μg/ml copper solution were pipetted into separatory funnels and the twice-distilled water was added to a volume of 25 ml. An equal volume of dilute sulfuric acid (1:20 diluted in twice-distilled water) was added. The copper was extracted by vigorous shaking for 60 sec with 2 ml of zinc dibenzyldithiocarbamate (0.05% w/v) in carbon tetrachloride. The copper complex solution was allowed to settle and the complex was drawn off and centrifuged to remove any turbidity.

Absorbance of all samples was determined in the visible at 435 mμ against a blank prepared by carrying equal volumes of the acid and water through the procedure. A linear relationship exists between absorbance at 435 mμ and copper levels up to about 8 μg. Some deviation occurs at higher concentrations and absorbance values decrease with increasing copper concentrations. Adjustment to the linear range can easily be accomplished by varying the volume of extracting reagent or by adjusting the amount of oil in the ashing step.

Preparation of Standards

Preparation of oils containing known amounts of added copper posed considerable difficulty. Samples containing copper added in the form of cupric oleate (commercial grade) gave values lower than the theoretical amount when analyzed by both activation and colorimetric methods. These results indicated that fatty copper soaps were not suitable in preparing standard oils with known levels of copper.

Addition of ethanolic solutions of copper salts to oils followed by magnetic stirring under a stream of nitrogen resulted in oils having erratic copper contents as duplicate determinations were in poor agreement.

To overcome these problems, pure copper nitrate prepared from copper metal turnings and nitric acid was used as the source of standard oils with known levels of copper. Suitable dilution of an ethanolic stock solution containing 1,000 μg/ml of copper were added to samples of soybean oil which were then steam deodorized at low temperature (19).

Two separate sets of standard oils calculated to contain 5.0, 1.0, 0.20 and 0.04 ppm of copper were prepared in this manner. The oils were then steam deodorized at low temperatures for 6 hr to remove the ethanol from the oil and to agitate the samples. This procedure gave the best results in overcoming the homogeneity problems since duplicate determinations were then in good agreement. However, oils so prepared gave consistent values which were slightly low for copper by both neutron activation and colorimetric methods (Table I). Since activation analysis requires no ashing step, this agreement between the two methods indicates dry ashing did not cause loss of copper by volatilization.

Copper Analysis With Zinc Dibenzyldithiocarbamate

The procedure followed is essentially that described by Abbot and Polhill (1) except smaller samples are used in ashing and 2 ml of colorimetric reagent instead of 10 ml. These modifications enhance the detection limits fivefold. After dry ashing, the residues are dissolved in 1:20 H_2SO_4 and the solution was made to a known volume in a volumetric flask. Aliquots are transferred to a separatory funnel and diluted with an equal volume of distilled H_2O. The copper is extracted for 60 sec with 2 ml of zinc dibenzyldithiocarbamate in carbon tetrachloride. The complex is allowed to settle, drawn off, and centrifuged to remove any turbidity in the sample. The adsorption is read at 435 mμ and the copper content determined from the standard absorption curve.

Results and Discussion

The effect of sample size on the dry-ashing rate of soybean oil is shown in Fig. 1. Sample sizes of 0.5, 1.5 and 2.5 per boat require, respectively, about 9, 24 and 27 hr, with oxygen flows of 30–50 ml/min and a forward power of 180–200 w. A 3-g sample, commonly employed, requires about 60–72 hr. Ashing is slow because a polymerized skin forms that resists oxidation. Oxidation can be increased by the addition of paper filter discs to the oil to expose a larger surface area to the oxidizing plasma. Problems were encountered, however, from contamination of the samples from copper in the filter discs.

TABLE I

Analysis of Soybean Oils Containing Added Copper by Colorimetry Activation, and Atomic Absorption

	Method		
	Colorimetric	Activation[a]	Atomic absorption[b]
Copper added, ppm		Copper found ppm[c]	
5.00	4.70	4.43	2.46
1.00	0.912	0.837	0.556
0.20	0.160	0.167	0.089
0.04	0.036	0.042	0.061

[a] Determined on neat oil.
[b] Oil diluted 1:4 in methyl isobutyl ketone.
[c] Corrected for copper content of starting oils. The first three levels (5.00, 1.00, 0.20 ppm) represent the mean of four determinations, the last level (0.04 ppm) represents the mean of six determinations for colorimetric and activation methods.

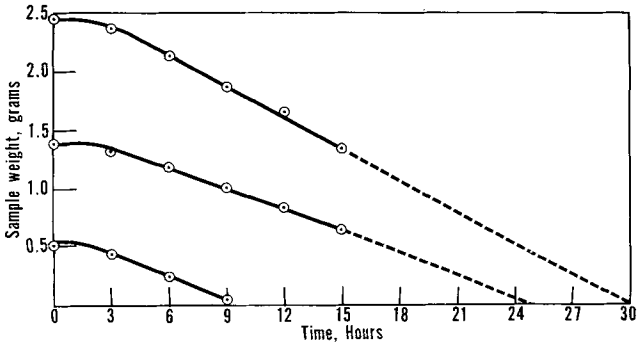

FIG. 1. Effect of sample size on the dry-ashing rate of soybean oil.

Samples containing 3–5 ppm copper require about 1–2 g of oil. For lower levels, such as 0.2 and 0.04 ppm, 9 to 15 g of oil must be used. Since sample weight is 3–5 g, residues from three to five boats must be combined before assay. Since these figures are approximate, they serve only as a guide for the copper analysis for various soybean oils by the colorimetric technique.

Table I shows the combined and averaged copper contents of two different soybean salad oils containing added copper at four levels as determined by colorimetric, neutron activation and atomic absorption methods. Data for each level (except the 0.04 ppm) represent the mean of four determinations by each method. Although data for both the activation and colorimetric methods are in fair agreement with the amount of copper added, the values are consistently low, which result could mean some copper was lost during deodorization and agitation.

Atomic absorption methods have been proposed for the determination of nearly every metal. Trace iron and copper in lubricating oils are commonly estimated by atomic absorption techniques (4). Applying these techniques to analysis of trace copper contents found in vegetable oils has posed more difficulties. Only two of six laboratories employing atomic absorption techniques have provided us with

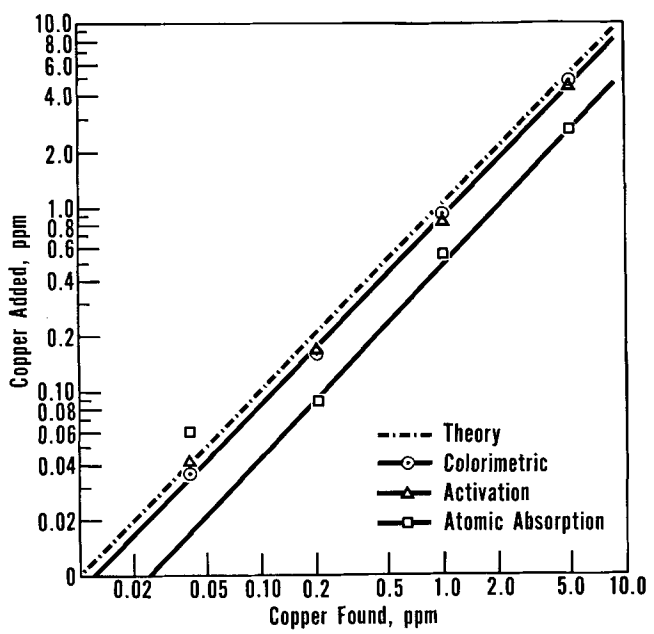

FIG. 2. Log-log plot of added copper vs. copper found by colorimetric, neutron activation and atomic absorption analyses.

TABLE II

Copper Contents of Some Natural and Copper Hydrogenated Soybean Oils

Sample	Oil	Method of Analysis	
		Colorimetric (ppm, copper)	Activation (ppm, copper)
A	Refined, bleached, deodorized	0.046 (3)[a]	0.058 (3)
B	Refined, bleached, deodorized	0.067 (3)	0.088 (3)
C	Refined and bleached	0.035 (2)	0.026 (1)
D	Refined and bleached	0.102 (1)	0.131 (1)
E	Copper-hydrogenated	3.21 (2)	3.25 (2)
F	Copper-hydrogenated	4.70 (2)	4.55 (1)
G	Copper-hydrogenated	4.14 (2)	3.82 (1)

[a] Figures in parentheses indicate number of determinations.

results that agree reasonably with other methods. Four of the laboratories were unable to quantitate copper contents of glyceride oils, some even after an ashing step had been performed. Two, however, obtained reasonably accurate results directly on a 1:4 dilution of the oil in methyl isobutyl ketone. Results were best when the standards used in calibrating the instrument had about the same viscosity as the vegetable oils themselves. Also, results were good with instruments having total consumption flame burners. Apparently the application of atomic absorption to trace copper analysis in vegetable oils requires rigorous standardization before yielding accurate data.

The atomic absorption data indicate that analyses are low by a factor of about 2, a level which is not readily explained. The amount of copper added versus the amount found by each method was plotted on log-log graph paper (Fig. 2). All three methods show a linear relationship between the amounts of copper added and copper found, except the 0.04 ppm level by atomic absorption. Since the slopes of the three lines, estimated by least squares, are in good agreement, evidently results are consistent by all three methods over the range 0.04–5.0 ppm added copper. A statistical evaluation of the analyses can be summarized as follows: Colorimetric and neutron activation methods give the same results; atomic absorption shows half the recovery of the other two methods; the relative error of a single determination is $\pm 13\%$ and that of the mean of duplicate determinations is $\pm 9\%$; i.e., 68.2% of the samples would be in error by less than $\pm 13\%$ or $\pm 9\%$ in duplicates; results are independent of the starting soybean salad oils; and when the ratio of two means based on duplicate determinations exceeds 1.31, the means are significantly different at the 0.95 probability level.

Table II shows the copper contents of some natural and copper hydrogenated soybean oils by colorimetric and activation analyses. Oils A, B, C and D are unhydrogenated, commercially processed, soybean oils; their copper contents vary from about 0.03–0.1 ppm. Oils E, F and G are oils hydrogenated with copper-containing catalysts; their copper contents increase from less than 0.1 to about 3–5 ppm after hydrogenation.

Five oil samples (E, F, H, I, J) were analyzed by four different laboratories (Table III). Good agreement exists among the first three laboratories for the copper contents of all samples. For some samples there is considerable disagreement between the data reported by one laboratory (given in the column atomic absorption II) and that reported by the other three laboratories. Of particular interest are oils I and J. Oil I is a reprocessed soybean oil. By reprocessed, we mean hydrogenated with a copper

TABLE III
Comparison of Methods for Copper Analysis in Soybean Oils

Sample	Oil	Method			
		Colorimetric Activation[a]	Atomic Absorption[b]		
			I	II	
		(ppm copper)			
E	Copper-hydrogented	3.21	3.25	3.36	1.60
F	Copper-hydrogenated	4.70	4.55	4.92	2.13
H	Refined, bleached, deodorized	0.035	0.026	0.04	0.05
I	Copper-hydrogenated, reprocessed[c]	0.039	0.039	0.02	0.10
J	Blend 10% E + 90% unhydrogenated H	0.29	0.35	0.27	0.29

[a] Determined on neat oil.
[b] Oil diluted 1:4 in methyl isobutyl ketone.
[c] See text.

catalyst, filtered, rebleached, treated with citric acid and deodorized. The copper content of this sample is low (0.04 ppm) and is equal, within experimental error, to that of the oil before hydrogenation. These data are preliminary, but in accord with the results of Dutton et al. (7) and of Beal et al. (3) who showed that nearly all copper can be removed from copper-hydrogenated soybean oil by certain processing techniques. Oil J is a blend of 10% E and 90% of a natural unhydrogenated soybean oil (H), and its analysis was performed as a further check of the methods used. The expected value for the sample as estimated from oil E (Table II) would be about 0.36 ppm, which is close to the value found by the various methods of analysis.

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